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Abstract. Microalgae is considered to be an important resource to address the global demand for sustainable energy and green technologies. Numerous applications of microalgae have already been identified in the past. They can be cultivated to produce food, animal feeds, nutraceuticals, and even biofuels. They can also be used for environmental applications such as carbon capture and storage, and wastewater treatment. There are different environmental factors that can affect the growth of microalgae such as light, nutrients, temperature, and aeration. Among different microalgae strains, *Chlorella sorokiniana* had been identified to be one of the most effective and commonly used strains across these different applications. In this study, the effect of aeration rate and light cycle on the growth characteristics of *C. sorokiniana* in a photobioreactor was investigated. Cultivation experiments were carried out at room temperature (24 – 26 ℃) under phototrophic conditions in which the light intensity was set to 150 μmol/m^2-s and the carbon source used was air enriched with carbon dioxide at 2.5% concentration. The aeration rates evaluated were 0.0125, 0.0250, 0.0500, 0.1000, and 0.2000 vvm while the light cycles evaluated were 24:0 (continuous illumination), and 12:12 (diurnal illumination). The results showed that in the 24:0 light cycle, increasing the aeration rate up to 0.1000 vvm led to an increase in the cumulative biomass production, specific growth rate, overall biomass productivity, and nitrate consumption of *C. sorokiniana* cultures. At 0.2000 vvm, no increase in any of these parameters were observed. Meanwhile, the aeration rate did not have any effect on the carbohydrate content of *C. sorokiniana*. On the other hand, cultivation under a 12:12 cycle resulted in a decrease in all of the parameters across all aeration rates evaluated. However, no significant interaction between the light cycle and the aeration rate was found in this study. Finally, among the conditions evaluated, the 24:0 light cycle and 0.1000 vvm aeration rate led to the best growth characteristics of *C. sorokiniana*. The results from this study indicate that aeration rate and light cycle have significant effects on cultivating microalgae such as *C. sorokiniana*. The results also showed that appropriate levels of these factors should be considered depending on the application of the microalgae cultivation. For future work, the growth of *C. sorokiniana* can be modeled to dynamically optimize these factors to improve its growth and reduce its cultivation costs.

1. Introduction

Microalgae has a lot of potential for several applications in food, animal feeds, nutraceuticals, biofuels, wastewater treatment, and CO_2 capture [1]. However, the cost of cultivating microalgae, especially in

photobioreactors is currently not competitive especially for some applications such as biofuel production [2]. Several studies suggested ways on how to reduce the cost of cultivating microalgae such as selecting and bioengineering microalgae strains, doing large-scale cultivation systems, and optimizing cultivation factors [3,4]. In this study, the focus is to better understand the effect of factors such as aeration and lighting on the growth characteristics of *Chlorella sorokiniana* in photobioreactors to gain possible insights on how to enhance the microalgae cultivation by controlling these factors.

Among microalgae strains, *Chlorella sorokiniana* is considered to be among the most productive species which can be used for food production, biofuels, and other high-valued products [5]. It is also a very resistant strain which is capable of growing at high temperatures and light intensity and removing high amounts of ammonia from wastewater [6].

There are several environmental factors to consider for microalgae cultivation such as aeration and lighting. Aeration supplies gas the culture and increases mass transfer, control toxic levels of dissolved oxygen and inhibitory levels of CO_2, avoid CO_2 deficiency, prevent sedimentation and more [7-10]. However, excessive aeration may damage microalgae cells and incur higher energy and CO_2 costs. Meanwhile, light is very important during phototrophic growth of microalgae for photosynthesis [11]. Sunlight is the most economical source of lighting for microalgae cultivation, but in some cases, there is still a need for artificial lighting, which may incur additional costs.

This study aims to assess the effect of aeration rate and lighting on the cultivation of *C. sorokiniana* and to understand the role of these factors in different applications of *C. sorokiniana* cultivation. In addition, this study may serve as the basis for modeling the growth of *C. sorokiniana* for simulations and dynamic optimization to improve its growth and reduce its cultivation costs.

2. Methodology

2.1. Microalgae strain and culture medium

The microalgae strain used, *C. sorokiniana* AK – 1, was provided by the Research Center for Energy Technology and Strategy in National Cheng Kung University. Seed cultures were prepared by inoculating 500 mL laboratory bottles containing the culture medium with small amounts of the *C. sorokiniana* AK – 1 which were initially isolated in agar plates. These seed cultures were grown at room temperature (i.e. 24 – 26 ℃) for 6 – 8 days. These were aerated with 2.5% carbon dioxide, illuminated at an intensity of 150 μmol/m^2-s with TL5 lamps (Philips Co., Taiwan) and agitated at 300 rpm using CIMAREC magnetic stirrers (Thermolyne, USA). BG – 11 was used as the culture medium for *C. sorokiniana* since it is a standard medium for growing *Chlorella sp.* [12]

2.2. Photobioreactor operation

Standard 1-L laboratory glass bottles were used for cultivating *C. sorokiniana* under phototrophic conditions. The volume of the growth medium used was 500 mL. The seed cultures that were initially prepared were then used to inoculate the photobioreactor with 18 – 22 mg L^{-1} of microalgae respectively. The cultures were grown at room temperature (i.e. 24 – 26 ℃) for 9 days. These were aerated with 2.5% carbon dioxide, illuminated at an intensity of 150 μmol/m^2-s with TL5 lamps (Philips Co., Taiwan), and agitated at 300 rpm using CIMAREC magnetic stirrers (Thermolyne, USA).

Aeration was provided via an air compressor (SV – 203, 2.2 Kw, Swan Air, Taiwan) coupled with a CO_2 tank. This assembly was connected to a gas mixing station that maintained the CO_2 concentration at 2.5%. The aeration rate going to the cultures were then controlled via flowmeters (RMA – 026-SSV, Dwyer, USA). Five aeration rates, i.e. 0.0125, 0.0250, 0.0500, 0.1000, and 0.2000 vvm were used to grow *C. sorokiniana* cultures under continuous light cycle(24:0). *C. sorokiniana* was also cultivated under a 12h light/12h dark or diurnal light cycle (12:12) at 0.0500, 0.1000, and 0.2000 vvm. During the dark periods, the lamps were closed and the photobioreactors were covered with black paper to prevent light from illuminating the microalgae cultures. Lastly, cultivation for each treatment was done at least in duplicates.

2.3. Determination of biomass and nitrate concentration

The biomass and nitrate concentrations (g L^{-1}) of the cultures were determined regularly by respectively measuring the optical density of the microalgae samples at 680 nm (OD_{680}) and their supernatants at 220 nm (OD_{220}) with a UV/VIS spectrophotometer. These wavelengths were also used by similar previous studies [13-15]. The samples were diluted to obtain an absorbance range between 0.05 – 0.9. The measured OD_{680} value of 163.1 mg L^{-1}/OD_{680} was converted to biomass concentration by using a pre-constructed calibration procedure. The same procedure was administered to quantify the nitrate concentration from the and measured OD_{220} value of 23.6 mg L^{-1}/OD_{220}.

2.4. Determination of carbohydrate content

The carbohydrate content was determined by adopting the modified quantitative saccharification method reported by [15]. Small amounts of dried microalgae (30 mg) were initially hydrolyzed by adding 3 mL of sulfuric acid (72%) and incubating them in a warm water bath in a period of 60 min. After this, the hydrolysates were diluted (2.5%) then autoclaved for the secondary hydrolysis. The hydrolysates were centrifuged and the supernatants were neutralized and analyzed using a high-performance liquid chromatography (L-2000 series, Hitachi, Japan). The concentrations of the sugars were calculated via extrapolation of measured peak areas and their pre-constructed calibration curves.

2.5. Statistical analysis

Different statistical tests such as Shapiro – Wilk W test and Levene test were first employed to check the statistical assumptions. After which, classical one-way ANOVA or Welch's test was used to check for significant differences. A confidence level of 95% was employed in all statistical analyses conducted using the JMP statistical software.

3. Results

3.1. Effect of aeration rate on the growth characteristics of C. sorokiniana

Figure 1 below shows the different time course profiles of the growth characteristics of *C. sorokiniana* at different aeration rates. In figure 1a, biomass concentration increased throughout the whole cultivation period and a normal pattern for microalgae growth under this type of cultivation (i.e. batch culture) was observed. Day 0 to 1 was the lag phase when the growth was still slow. Day 1 to 4 was the exponential phase where the cultures experienced rapid growth. Then day 4 to 9, the growth started to slow down as the cultures entered the phase of declining relative growth. This is similar to the growth curves of *C. sorokiniana* in the study of [12]. From the same figure, the final biomass concentration increased as the aeration rate increased. The increase in biomass concentration became smaller as the aeration rate increased which was evident between the 0.1000 vvm and 0.2000 vvm profiles. This indicates that it may not be cost-effective to further increase aeration beyond this level, at least for this cultivation setup.

From figure 1b, pH level was lower for higher aeration rates which can be partially attributed to the formation of carbonic acid from CO_2 addition. From this, the steep drop in pH during the first day for 0.1000 vvm and 0.2000 vvm can be explained as a result of low CO_2 utilization due to the low microalgae concentration at the start of the cultivation. These findings on pH level agree with the findings of [12], where the addition of more CO_2 was actually used to decrease the pH of their microalgae cultures. At high pH levels, biomass production was lower due to the limitation in CO_2. Meanwhile, too much CO_2 was utilized inefficiently and was deemed as uneconomical.

As expected, nitrate concentration decreased throughout the cultivation period as shown in figure 1c. In addition, it can be observed in the same figure that nitrate consumption was fastest during the time exponential phase and similarly it started to slow down when the microalgae cultures entered the phase of declining relative growth. This shows the nitrate consumption reflected the increase in biomass.

Results from statistical tests show a reported significant difference on the cumulative biomass production, specific growth rate, overall biomass productivity and nitrate consumption of the *C. sorokiniana* cultures grown at different aeration rates ($p < 0.05$). Specifically, cultivation at 0.1000 vvm aeration rate led to the highest cumulative biomass production (2.543 g/L), sp. growth rate (0.542 d^{-1}), overall biomass productivity (280.37 mg L^{-1} d^{-1}) and nitrate consumption (76.46%). Meanwhile, aeration

rate had no effect on the carbohydrate content (i.e. $p > 0.05$), which on average, composed 27.94% of the dried microalgae mass.

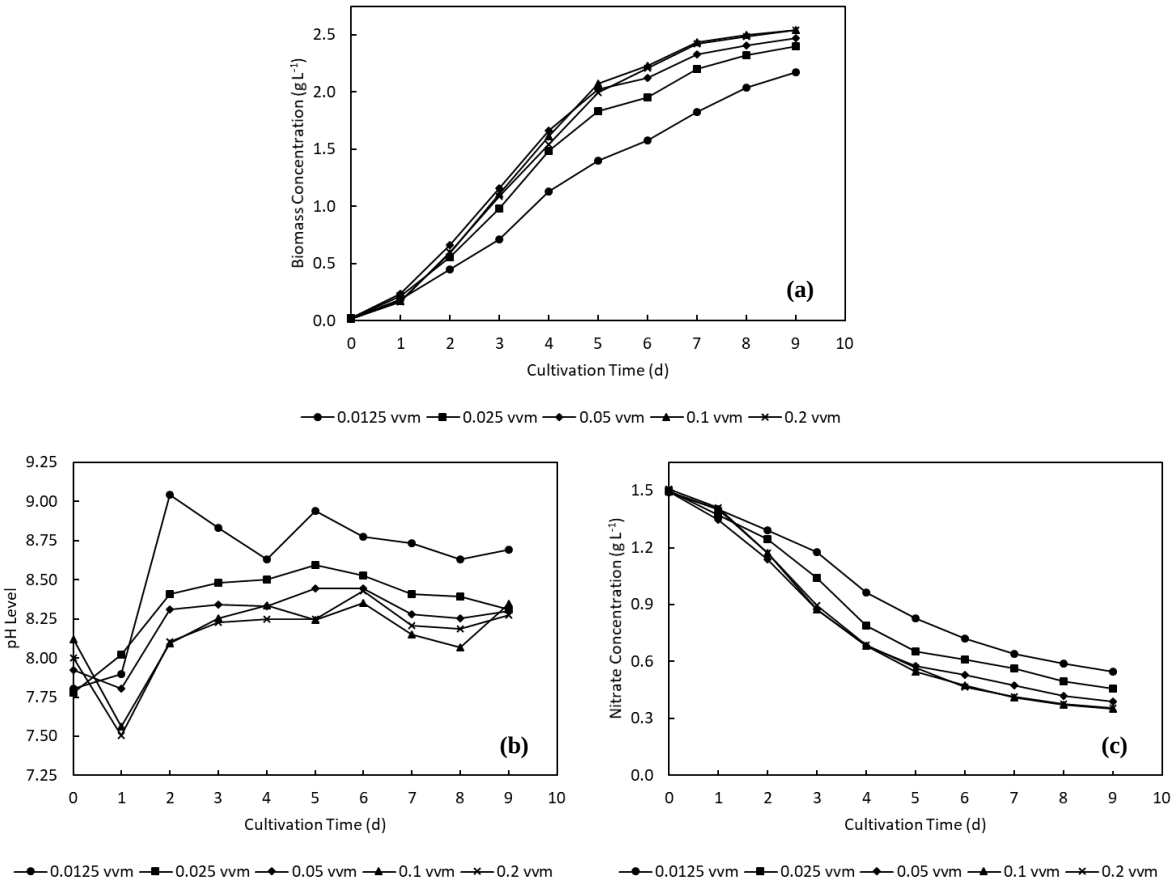

Figure 1. Biomass concentration (a), pH level (b), and nitrate concentration (c) of *C. sorokiniana* at different aeration rates and continuous lighting at 24h periods (CO_2 concentration: 2.5 %; light intensity: 150 μmol/m^2-s)

3.2. Effect of light cycle on the growth characteristics of C. sorokiniana

Figure 2 below shows the time course profiles of biomass concentration and pH level of *C. sorokiniana* under a 12:12 cycle. In figure 2a, biomass concentration generally increased throughout the whole cultivation period. However, biomass losses were observed after the dark periods.

From figure 2b, the pH level of the cultures grown under a 12:12 cycle went down steeply after the first day due to low CO_2 utilization from low initial biomass concentration. Also, as seen in the same figure, there are small increases and drops in the pH level throughout the cultivation period. The increase in pH was due to the uptake of CO_2 for photosynthesis during the light period. Meanwhile, the drop in pH was due to low CO_2 utilization during the dark period. Similar findings were reported in the study of [16] where the pH levels of carbon limited microalgae ponds were monitored.

As seen in figure 3a, nitrate consumption was still high even in under a 12:12 cycle, which only went down by around 15% compared in continuous lighting. This indicates that *C. sorokiniana* is still effective in removing nitrates even under a 12:12 cycle. This ability suits applications such as wastewater treatment in which high nitrate removal rate is important. Meanwhile, carbohydrate content was lower by around 50% under a 12:12 cycle as shown in figure 3b. This is most likely due to microalgae cells respiring their carbohydrate reserves for maintenance during dark periods [17]. Thus for applications which require high carbohydrate (glucose) content, (e.g. bioethanol production) it may be suggested to maximize the light period to maximize carbohydrate (glucose) production and preservation.

Results from statistical tests show a reported significant difference on the cumulative biomass production, specific growth rate, overall biomass productivity and nitrate consumption of the *C. sorokiniana* cultures grown at different light cycles ($p < 0.05$). However, there is no reported significant difference found in any of the growth parameters mentioned at different interaction levels of aeration rates and lighting conditions. In addition to these, the data gathered can be used to create growth models of *C. sorokiniana*. These can be used to dynamically optimize aeration rate and light cycles to improve its growth and reduce cultivation costs. More importantly, the results were able to provide some basis for selecting appropriate conditions for applications of *C. sorokinina* cultivation such as wastewater treatment and biofuel production in terms of lighting and aeration.

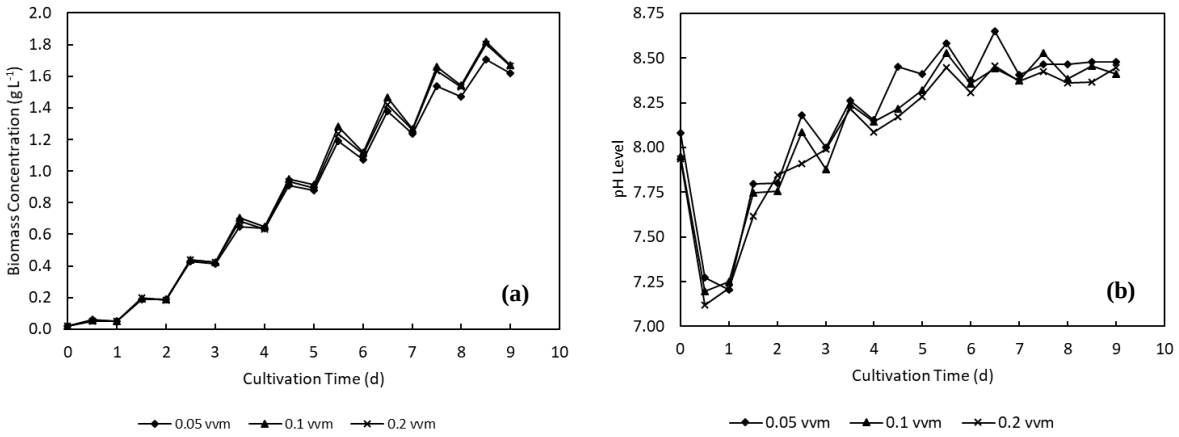

Figure 2. Biomass concentration (a), and pH level (b) of *C. sorokiniana* at different aeration rates and 12:12 cycle at 12h periods (CO_2 concentration: 2.5 %; light intensity: 150 µmol/m^2s).

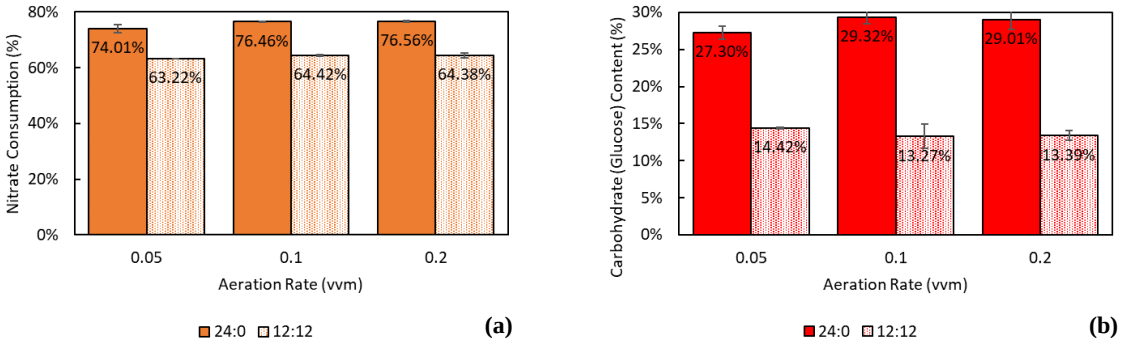

Figure 3. Total nitrate consumption (a), and carbohydrate content (b) of *C. sorokiniana* at different aeration rates and light cycles.

4. Conclusion

This study investigated and demonstrated the effects of aeration rate and light cycle on the growth characteristics of *Chlorella sorokiniana* in a photobioreactor. Results showed that cumulative biomass concentration, sp. growth rate, overall biomass productivity, and nitrate consumption were influenced by the aeration rate and were highest at 0.1000 vvm during continuous lighting. Meanwhile, carbohydrate content was not affected by aeration rate. On the other hand, the light cycle had statistically significant effects on all of these parameters which all went down under a 12:12 cycle. Most notably, carbohydrate content went down by almost 50% due to carbohydrate (glucose) respiration during dark periods. Nitrate consumption also went down but only for around 15%, suggesting that *C. sorokiniana* may be still effective in removing nitrates even under non-continuously lighted conditions. These results show that both the aeration rate and light cycle have significant effects on cultivating microalgae such as *C. sorokiniana*. In addition, these results showed how aeration rate and light cycle affect microalgae cultivation for different applications. For future work, the growth of *C. sorokiniana* can be modeled to

dynamically optimize aeration and light cycles to improve its growth and potentially reduce cultivation costs.

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